IN THE SPECIFICATION

Please replace the Abstract with the following:

The present invention relates to genes, proteins and methods comprising carotenoid monooxygenases in the cytochrome P450 family. Specifically, the invention provides plants with altered carotenoid ratios using an *Arabidopsis LUT1* gene for altering monooxygenase P450 activity. In a preferred embodiment, the present invention relates to altering carotenoid ratios in plants and microorganisms using LUT1 .epsilon.-hydroxylases and/or CYP97A .beta.-hydroxylases derived from plants.

Please replace paragraph [0156] with the following:

[0156] The *LUT1* locus has previously been mapped to the bottom arm of chromosome 3 at 67 ± 3 cM (Tian, *et al. Plant Mol. Biol.* 47, 379-388 (2001), herein incorporated by reference). For fine mapping of the locus, 530 plants homozygous for the *lut1* mutation were identified from approximately 2,000 plants in a segregating F_2 mapping population. Using SSLP markers, *LUT1* was initially localized to an interval spanning two BAC clones (F8J2 and T4D2) and was further delineated to a 100 kb interval containing 30 predicted proteins (Fig. 2*A*). The term "BAC" and "bacterial artificial chromosome" refers to a vector carrying a genomic DNA insert, typically 100-200 kb. The term "SSLP" and "simple sequence length polymorphisms" refers to a unit sequence of DNA (2 to 4 bp) that is repeated multiple times in tandem wherein common examples of these in mammalian genomes include runs of dinucleotide or trinucleotide repeats (for example, CACACACACACACACACA (SEQ ID NO:59)." As with all other carotenoid biosynthetic enzymes, the *LUT1* gene product is predicted to be chloroplast-targeted and within the 100 kb interval containing *LUT1*, six proteins were predicted as being chloroplast-targeted by the TargetP prediction software (Emanuelsson et al., (2000) J. Mol. Biol., 300: 1005-1016 and Henrik et al., (1997) Protein Engineering, 10:1-6).(~~http://~~www.cbs.dtu.dk/services/TargetP). One of these chloroplast-targeted proteins, At3g53130, is a member of the cytochrome P450 monooxygenase family (CYP97C1). Cytochrome P450 monooxygenases are heme-binding proteins that insert a single oxygen

atom into substrates, e.g. hydroxylation reactions, and therefore At3g53130 was considered to be a strong candidate for *LUT1*.

Please replace paragraph [0162] with the following:

[0162] Genomic DNA from homozygous *lut1* F_2 plants was isolated using the DNAzol reagent following the manufacturer's instructions (Invitrogen, Carlsbad, CA). PCR reactions were performed with 1 µl of genomic DNA in a 20 µl reaction mixture. The PCR program was 94° C for 3 min, 60 cycles of 94° C for 15 s, 50° C-60° C (the annealing temperature was optimized for each specific pair of primers) for 30 s, 72° C for 30 s, and finally 72° C for 10 min. A portion of the PCR product was then separated on a 3% agarose gel. *lut1* had been previously mapped to 67 ± 3 cM on chromosome 3 (Tian, *et al. Plant Mol. Biol.* 47, 379-388 (2001). Simple Sequence Length Polymorphism (SSLP) markers for fine mapping in this interval were designed based on the insertions/deletions (INDELs) information obtained from the Monsanto website: ~~http://~~www.arabidopsis.org/Cereon/.

Please replace paragraph [0168] with the following:

[0168] The deduced amino acid sequence of ***LUT1*** contains several features characteristic of cytochrome P450 enzymes (FIG. 2C). Cytochrome P450 monooxygenases contain a consensus sequence of (A/G)GX(D/E)T(T/S) that forms a binding pocket for molecular oxygen with the invariant Thr residue playing a critical role in oxygen binding in both prokaryotic and eukaryotic cytochrome P450s (Chapple, Annu. Rev. Plant Physiol. Plant Mol. Biol. 49, 311-343 ~~(1998,~~(1998) herein incorporated by reference). In the deduced ***LUT1*** protein sequence, this oxygen-binding pocket is highly conserved (single underlined amino acids in FIG. 2C). The conserved sequence around the heme-binding cysteine residue for cytochrome P450 type enzymes is FXXGXXXCXG, and is also present in ***LUT1*** (double underlined amino acids in FIG. 2C).

Please replace paragraph [0169] with the following:

[0169] The chloroplast transit peptide prediction software ChloroP v 1.1

(~~http://~~www.cbs.dtu.dk/services/ChloroP/) predicts an N-terminal transit peptide in LUT1 that is cleaved between Arg-36 and Ser-37 (Fig. 2*C*). The predicted chloroplast localization for LUT1 is consistent with the subcellular localization of carotenoid biosynthesis in higher plants (Cunningham and Gantt, *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 49, 557-583 (1998)) but is uncommon for a plant cytochrome P450. Out of the 272 predicted cytochrome P450s in the Arabidopsis genome, only nine, including LUT1, are predicted to be chloroplast-targeted (Schuler and Werck-Reichhart, *Annu. Rev. Plant Biol.* 54, 629-667 (2003), herein incorporated by reference). LUT1 also contains a single predicted transmembrane domain (shaded box, Fig. 2*C*), which contrasts with the four transmembrane domains predicted for the non-heme di-iron β-hydroxylases (Cunningham and Gantt, *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 49, 557-583 (1998), herein incorporated by reference). Initial attempts to express and assay LUT1 protein in yeast were unsuccessful.

Please replace paragraph [0174] with the following:

[0174] Our *Arabidopsis* LUT1 sequence was previously designated as CYP97C1 according to the standardized cytochrome P450 nomenclature (~~http://~~www.biobase.dk/P450). The *Arabidopsis* genome also contains two other CYP97 family members, CYP97A3 and CYP97B3, which are 49% and 42% identical to the LUT1 polypeptide, respectively. Interestingly, CYP97A3 (At1g31800) is also one of the nine cytochrome P450s in Arabidopsis predicted to be chloroplast-targeted, while CYP97B3 (At4g15110) is predicted to be targeted to the mitochondria (Schuler and Werck-Reichhart, *Annu. Rev. Plant Biol.* 54, 629-667 (2003), herein incorporated by reference). Additional CYP97 family proteins were identified in the EST and genomic databases from a wide variety of monocots and dicots, including *Arabidopsis*, barley, rice, wheat, soybean, pea, sunflower, tomato, and diatom (Figs. 5 and 8). The term "EST" and "expressed sequence tag" refers to a unique stretch of DNA within a coding region of a gene; approximately 200 to 600 base pairs in length. The term "contig" refers to an overlapping collection of sequences or clones.

Please replace paragraph [0326] with the following:

[0326] Genomic DNA from homozygous *lut1* F_2 plants was isolated using the DNAzol reagent following the manufacturer's instructions (Invitrogen, Carlsbad, CA). PCR reactions were performed with 1 µl of genomic DNA in a 20 µl reaction mixture. The PCR program was 94° C for 3 min, 60 cycles of 94° C for 15 s, 50° C-60° C (the annealing temperature was optimized for each specific pair of primers) for 30 s, 72° C for 30 s, and finally 72° C for 10 min. A portion of the PCR product was then separated on a 3% agarose gel. *lut1* had been previously mapped to 67 ± 3 cM on chromosome 3 (Tian, *et al. Plant Mol. Biol.* 47, 379-388 (2001)). Additional Simple Sequence Length Polymorphism (SSLP) markers for fine mapping in this interval were designed based on the insertions/deletions (INDELs) information obtained from the Monsanto website: ~~http://~~www.arabidopsis.org/Cereon/.

Please replace paragraph [0328] with the following:

[0328] Isolation of T-DNA Knockout Mutants in At3g53130 and Generation of a Carotenoid Hydroxylase Triple Knockout Mutant Line. At3g53130 specific primers (forward, 5'-CTTCCTCTTCTTACTCTTCTCTCTTCACT-3' (SEQ ID NO:28); reverse, 5'-AAGAACGATGGATGTTATAGACTGAAATC-3' (SEQ ID NO:29)) were sent to the University of Wisconsin Arabidopsis T-DNA knockout facility to identify knockout mutants of the *LUT1* gene. A single knockout line, designated *lut1-3*, was identified and isolated as described (~~http://~~www.biotech.wisc.edu/Arabidopsis/). In order to generate a hydroxylase triple knockout mutant line, homozygous *lut1-3* and *b1 b2* plants were crossed. Putative *lut1-3 b1 b2* triple mutants were identified from the segregating F_2 population by HPLC and their genotypes confirmed by PCR as previously described (Tian, *et al. Plant Cell* 15, 1320-1332 (2003), herein incorporated by reference).

Please replace paragraph [0330] with the following:

[0330] Phylogenetic Analysis of LUT1 Homologs. Full-length protein sequences of putative LUT1 homologs from *Arabidopsis thaliana*, *Glycine max*, *Oryza sativa*, and *Pisum sativum* were obtained from GenBank: CYP97A3 (AAL08302), CYP97B1 (CAA89260), CYP97B2 (AAB94586), CYP97B3 (CAB10290), CYP97C1 (AAM13903), CYP97C2 (AAK20054) and CYP86A8 (CAC47665). Rice CYP97A4 and

CYP97B4 sequences were obtained from the cytochrome P450 website (~~http://~~drnelson.utmem.edu/CytochromeP450.html).

Please replace paragraph [0331] with the following:

[0331] Additional plant LUT1 homologs were retrieved from The Institute of Genome Research (TIGR) Unique Gene Indices: TC76166 (*Hordeum vulgare*), TC163981 (*Glycine max*), and TC69886 (*Hordeum vulgare*). The coding sequences of each were extracted, assembled, and corrected by the ESTscan program (~~http~~://tigrblast.tigr.org/tgi/). Chlamydomonas CYP97A3 homolog (Scaffold1399) was obtained from the DOE Joint Genome Institute (JGI) database (~~http://~~genome.jgi-psf.org/chlre1/chlre1.home.html). The term "scaffold" refers to a result of connecting contigs by linking information from paired-end reads from plasmids, paired-end reads from BACs, known messenger RNAs or other sources. The contigs in a scaffold are ordered and oriented with respect to one another and sometimes refered to as s supercontig. The term "supercontig" refers to a contig formed when an association can be made between two contigs that have no sequence overlap. This commonly occurs using information obtained from paired plasmid ends. For example, when both ends of a BAC clone are sequenced and it can be inferred that these two sequences are approximately 150-200 Kb apart (based on the average size of a BAC), then further if the sequence from one end is found in a particular sequence contig, and the sequence from the other end is found in a different sequence contig, the two sequence contigs are said to be linked. Truncated LUT1 homologs from *Zea mays*, lettuce, and cotton are also present in the databases but were not used for phylogenetic analysis because full-length assemblies were not possible.

Please replace paragraph [0334] with the following:

[0334] The *LUT1* locus has previously been mapped to the bottom arm of chromosome 3 at 67 ± 3 cM (Tian, *et al. Plant Mol. Biol.* 47, 379-388 (2001), herein incorporated by reference). For fine mapping of the locus, 530 plants homozygous for the *lut1* mutation were identified from approximately 2,000 plants in a segregating F_2 mapping population. Using SSLP markers, *LUT1* was initially localized to an interval spanning two BAC

clones (F8J2 and T4D2) and was further delineated to a 100 kb interval containing 30 predicted proteins (Fig. 2*A*). As with all other carotenoid biosynthetic enzymes, the *LUT1* gene product is predicted to be chloroplast-targeted and within the 100 kb interval containing *LUT1*, six proteins were predicted as being chloroplast-targeted by the TargetP prediction software (~~http://~~www.cbs.dtu.dk/services/TargetP). One of these chloroplast-targeted proteins, At3g53130, is a member of the cytochrome P450 monooxygenase family (CYP97C1). Cytochrome P450 monooxygenases are heme-binding proteins that insert a single oxygen atom into substrates, *e.g.* hydroxylation reactions, and therefore At3g53130 was considered to be a strong candidate for *LUT1*.

Please replace paragraph [0339] with the following:

[0339] The chloroplast transit peptide prediction software ChloroP v 1.1 (~~http://~~www.cbs.dtu.dk/services/ChloroP/) predicts an N-terminal transit peptide in LUT1 that is cleaved between Arg-36 and Ser-37 (Fig. 2*C*). The predicted chloroplast localization for LUT1 is consistent with the subcellular localization of carotenoid biosynthesis in higher plants (Cunningham and Gantt, *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 49, 557-583 (1998), herein incorporated by reference) but is uncommon for a plant cytochrome P450. Out of the 272 predicted cytochrome P450s in the Arabidopsis genome, only nine, including LUT1, are predicted to be chloroplast-targeted (Schuler and Werck-Reichhart, *Annu. Rev. Plant Biol.* 54, 629-667 (2003), herein incorporated by reference). LUT1 also contains a single predicted transmembrane domain (shaded box, Fig. 2*C*), which contrasts with the four transmembrane domains predicted for the non-heme di-iron β-hydroxylases (Cunningham and Gantt, *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 49, 557-583 (1998), herein incorporated by reference). Initial attempts to express and assay LUT1 protein in yeast were unsuccessful.

Please replace paragraph [0342] with the following:

[0342] Arabidopsis LUT1 was previously designated as CYP97C1 according to the standardized cytochrome P450 nomenclature (~~http://~~www.biobase.dk/P450). The Arabidopsis genome also contains two other CYP97 family members, CYP97A3 and CYP97B3, which are 49% and 42% identical to the LUT1 protein, respectively.

Interestingly, CYP97A3 (At1g31800) is also one of the nine cytochrome P450s in Arabidopsis predicted to be chloroplast-targeted, while CYP97B3 (At4g15110) is predicted to be targeted to the mitochondria (Schuler and Werck-Reichhart, *Annu. Rev. Plant Biol.* 54, 629-667 (2003), herein incorporated by reference). Additional CYP97 family proteins were identified in the EST and genomic databases from a wide variety of monocots and dicots, including Arabidopsis, barley, rice, soybean, and pea (Fig. 5).